

CLAIMS

1. A method of providing a polypeptide preparation having a reduced content of undesired enzymatic side activities, the method comprising the steps of:

5

(i) providing a medium having a pH of 2.0 or higher that comprises at least one desired polypeptide and in addition hereto at least one undesired enzymatic side activity, and

(ii) subjecting said medium to a pH of less than 2.0 for a period of time that is sufficient to

10 at least partially inactivate the at least one enzymatic side activity.

2. A method according to claim 1 wherein at least 75% of the activity of the at least one desired polypeptide is retained after subjecting the medium having a pH of 2.0 or more to a pH of less than 2.0.

15

3. A method according to claim 2 wherein at least 85% of the activity of the at least one desired polypeptide is retained.

4. A method according to any of claims 1-3 wherein at least 50% of the activity of the at 20 least one undesired enzymatic activity is inactivated.

5. A method according to claim 4 wherein at least 90% of the activity of the at least one undesired enzymatic activity is inactivated.

25-6. A method according to any of claims 1-5 wherein the medium having a pH of 2.0 or higher is a medium derived from the cultivation of an organism that during its cultivation produces the at least one desired polypeptide and the at least one undesired enzymatic side activity.

30-7. A method according to any of claims 1-6 wherein the at least one desired polypeptide is selected from the group consisting of an enzyme, an antibody, an antigen and a pharmaceutically active polypeptide.

8. A method according to any of claims 1-7 wherein the at least one enzymatic side activity is selected from the group consisting of glucoamylase activity, starch degrading en-

zyme activity, protease activity, peptidase activity, phosphatase activity, lipase activity, cellulase activity, lactase activity and hemicellulase activity.

9. A method according to any of claims 1-8 wherein the medium having a pH of 2.0 or
5 higher is derived from the cultivation of an organism that is selected from the group
consisting of an animal species, a plant species, a bacterial species, a yeast species and
a species of filamentous fungi.

10. A method according to claim 9 wherein the bacterial species is selected from the
10 group consisting of a gram negative bacterial species including *E. coli* and a gram positive
species including a *Bacillus* species.

11. A method according to claim 9 wherein the yeast species is selected from the group
consisting of *Saccharomyces cerevisiae*, a methyotrophic yeast species including *Pichia*
15 *pastoris* and a *Kluyveromyces* species including *Kluyveromyces lactis*.

12. A method according to claim 9 wherein the species of filamentous fungi is selected
from the group consisting of an *Aspergillus* species, a *Cyphomycetes* species, a *Fusarium*
species, a *Rhizomucor* species and a *Trichoderma* species.

20 13. A method according to any of claims 1-12 wherein the medium having a pH of 2.0 or
higher is subjected to a pH in the range of 1.0 to 1.99.

14. A method according to claim 13 wherein the pH is in the range of 1.5 to 1.99.

25 15. A method according to claim 14 wherein the pH is in the range of 1.7 to 1.99.

16. A method according to claim 15 wherein the pH is about 1.8.

30 17. A method according to any of claims 13-16 wherein the pH in the range of 1.0 to 1.99
is provided by adding an inorganic or an organic acid.

18. A method according to any of claims 1-17 wherein the medium having a pH of 2.0 or
higher is subjected to a pH of less than 2.0 for a period of time that is in the range of 0.1
35 minutes to 48 hours.

19. A method according to any of claims 1-18 wherein the at least one desired polypeptide has aspartic protease activity.

5 20. A method according to claim 19 wherein the medium having a pH of 2.0 or higher is a medium derived from the cultivation of a microorganism that during the cultivation produces the aspartic protease and the at least one undesired enzymatic side activity.

10 21. A method according to claim 20 wherein the medium is derived from the cultivation of a microorganism that naturally produces the aspartic protease or from the cultivation of a recombinant microorganism that has an inserted gene expressing the aspartic protease.

15 22. A method according to claim 21 wherein the microorganism is selected from the group consisting of a bacterial species, a yeast species and a species of filamentous fungi.

23. A method according to claim 22 wherein the aspartic protease is expressed as a fusion protein having, in addition to the aspartic protease activity, at least one undesired enzymatic side activity.

20 24. A method according to claim 23 wherein the at least one enzymatic side activity is starch-degrading enzyme activity including an activity selected from the group consisting of amylase activity and glucoamylase activity.

25. A method according to any of claims 20-24 wherein the microorganism is one that naturally produces at least one enzymatic side activity.

30 26. A method according to claim 25 wherein the at least one enzymatic side activity is selected from the group consisting of glucoamylase activity, lactase activity, starch degrading enzyme activity, protease activity, peptidase activity, phosphatase activity, lipase activity, cellulase activity and hemicellulase activity.

35 27. A method according to any of claims 19-26 wherein the aspartic protease is derived from the group consisting of an animal aspartic protease including a mammalian aspartic protease, a plant aspartic protease and a microbial aspartic protease.

28. A method according to claim 27 wherein the mammalian aspartic protease is selected from the group consisting of pro-chymosin, chymosin, pepsinogen and pepsin.

29. A method according to claim 28 wherein the aspartic protease is derived from a 5 mammalian species selected from the group consisting of a ruminant species, a *Camelidae* species including *Camelus dromedarius*, a porcine species, an *Equidae* species and a primate species.

30. A method according to claim 29 wherein the ruminant species is selected from the 10 group consisting of a bovine species, an ovine species, a caprine species, a deer species, a ~~buffalo species~~, an antelope species and a giraffe species.

31. A method according to any of claims 27-30 wherein the mammalian derived aspartic protease is a protease naturally produced in a mammalian species.

15 32. A method according to claim 27 wherein the aspartic protease is derived from a naturally produced aspartic protease by the addition or deletion of one or more amino acids or substitution of one or more amino acids herein.

20 33. A milk clotting composition comprising a preparation of an aspartic protease, provided by the method of any of claims 1-32, said composition essentially not having undesired enzymatic side activities.

34. A composition according to claim 33 essentially not having an undesired enzymatic 25 side activity selected from the group consisting of glucoamylase activity, lactase activity, starch degrading enzyme activity, protease activity, peptidase activity, phosphatase activity, lipase activity, cellulase activity and hemicellulase activity.